

CLAIMS

1. A purified decoy probe comprising,  
a first nucleotide base recognition sequence region, wherein said first region binds  
5 to an RNA polymerase, and  
an optionally present second nucleotide base recognition sequence region,  
provided that if said first region is nucleic acid, then said second region is either  
directly joined to the 5' end of said first region or is joined to the 3' end or 5' end of said  
first region by a non-nucleotide linker,  
10 wherein said optionally present second region is present if said first region can be  
used to produce a functional double-stranded promoter sequence using a complementary  
oligonucleotide,  
further provided that if said first region is nucleic acid which can be used to  
produce said functional double-stranded promoter sequence using said complementary  
15 oligonucleotide, then said decoy probe does not have a nucleic acid sequence greater than  
about 10 nucleotides in length joined directly to the 3' end of said first region.
2. The decoy probe of claim 1, wherein said first region is nucleic acid, said  
second region is directly joined to the 5' end of said first region, and said decoy probe does  
20 not have a nucleotide base sequence greater than 10 nucleotides in length joined directly to  
its 3' end.
3. The probe of claims 1, wherein said probe consists of 15 to 100 optionally  
modified nucleosides and one or more blocking groups located at the 3' terminus of said  
25 probe, wherein each of said optionally modified nucleosides independently has,  
a purine or pyrimidine moiety independently selected  
from the group consisting of inosine, uracil, adenine, guanine, thymine and  
cytosine; and  
a sugar moiety independently selected from the group  
30 consisting of deoxyribose, 2'-methoxy ribose, and ribose; and

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each of said optionally modified nucleosides is joined together by an internucleoside linkage independently selected from the group consisting of phosphodiester, phosphorothioate, and methylphosphonate.

- 5           4.       The probe of claim 3, wherein  
              at least 80% of said optionally modified nucleosides have a purine or pyrimidine  
              moiety independently selected from the group consisting of adenine, guanine, thymine and  
              cytosine, and a deoxyribose sugar moiety; and  
              at least 80% of said internucleoside linkages joining said optionally modified  
10       nucleosides are phosphodiester.
5.       The probe of claim 4, wherein said probe consists of 15 to 100  
              independently selected deoxyribonucleotides and one or more blocking groups located at  
              the 3' terminus of said probe.
- 15           6.       The probe of claim 3, wherein said one or more blocking groups are  
              selected from the group consisting of phosphorothioate, alkane-diol residue, cordycepin,  
              and an alkyl group.
7.       The probe of claim 6, wherein said probe consists of 35 to 70  
              independently selected nucleotides, said one or more blocking groups, and said second  
20       region comprises at least 10 nucleotides.
8.       The probe of claim 7, wherein said RNA polymerase is T7 RNA  
              polymerase.
9.       The probe of claim 7, wherein said RNA polymerase is T3 RNA  
              polymerase.
- 25           10.       The probe of claim 7, wherein said RNA polymerase is SP6 RNA  
              polymerase.

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11. A purified decoy probe comprising,

a first nucleotide base recognition sequence region, wherein said first region has at least 35% sequence similarity to an RNA polymerase promoter sequence, and

an optionally present second nucleotide base recognition sequence region,

5 provided that if said first region is nucleic acid, then said second region is either directly joined to the 5' end of said first region or is joined to the 3' end or 5' end of said first region by a non-nucleotide linker,

wherein said optionally present second region is present if said first region can be used to produce a functional double-stranded promoter sequence using a complementary  
10 oligonucleotide,

further provided that if said first region is nucleic acid which can be used to produce said functional double-stranded promoter sequence using said complementary oligonucleotide, then said decoy probe does not have a nucleic acid sequence greater than about 10 nucleotides in length joined directly to the 3' end of said first region.

15 12. The decoy probe of claim 11, wherein said first region is nucleic acid, said second region is directly joined to the 5' end of said first region, and said decoy probe does not have a nucleotide base sequence greater than 10 nucleotides in length joined directly to its 3' end.

20 13. The probe of claim 11, wherein said probe consists of 15 to 100 optionally modified nucleosides and one or more blocking groups located at the 3' terminus of said probe, wherein each of said optionally modified nucleosides independently has,

a purine or pyrimidine moiety independently selected

from the group consisting of inosine, uracil, adenine, guanine, thymine and cytosine; and

25 a sugar moiety independently selected from the group

consisting of deoxyribose, 2'-methoxy ribose, and ribose; and

each of said optionally modified nucleosides is joined together by an internucleoside linkage independently selected from the group consisting of phosphodiester, phosphorothioate, and methylphosphonate.

14. The probe of claim 13, wherein  
at least 80% of said optionally modified nucleosides has a purine or pyrimidine  
moiety independently selected from the group consisting of adenine, guanine, thymine and  
cytosine, and a deoxyribose sugar moiety; and

5 at least 80% of said internucleoside linkages joining said optionally modified  
nucleosides are phosphodiester.

15. The probe of claim 14, wherein said probe consists of 35 to 70  
independently selected nucleotides, one or more blocking groups, and said second region  
10 comprises at least 10 nucleotides.

16. The probe of claim 13, wherein said one or more blocking groups are  
selected from the group consisting of phosphorothioate, alkane-diol residue, cordycepin,  
and an alkyl group.

15 17. The probe of claim 16, wherein said first region has a nucleotide base  
sequence similarity of at least 75% with at least one of SEQ ID Nos.1, 2, 3, 4, 5 and 6.

18. The probe of claim 17, wherein said first region has a sequence similarity  
20 of 75% to 95% with SEQ ID NO: 3.

19. A reagent mixture for use in an amplification reaction comprising a nucleic  
acid polymerase and a reversible inhibitor of said polymerase, wherein said reagent  
mixture does not contain a nucleic acid substantially complementary to said inhibitor.

25 20. The reagent mixture of claim 19, wherein said reagent mixture does not  
contain an oligonucleotide having a 3' OH available for a primer extension reaction, and  
said inhibitor is not a substrate in a primer extension reaction.

30 21. The reagent mixture of claim 19, wherein said nucleic acid polymerase is  
an RNA polymerase.

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22. The reagent mixture of claim 21, wherein said inhibitor is a decoy probe comprising a nucleotide base recognition sequence having at least 35% sequence similarity to an RNA polymerase promoter sequence.

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23. A method for amplifying a target nucleic acid sequence under amplification conditions comprising the steps of:

- a) producing a mixture comprising an amplification enzyme and a reversible inhibitor of said enzyme, wherein said reversible inhibitor does not hybridize to a target nucleic acid comprising said target nucleic acid sequence under said amplification conditions, and wherein said mixture does not contain said target nucleic acid; and
- b) providing said mixture to said target nucleic acid, and
- c) amplifying said target nucleic acid sequence under said amplification conditions.

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24. The method of claim 23, wherein said method is a transcription-associated amplification, prior to said step (b) no amplification oligonucleotides used in said transcription-associated amplification are present, and said amplification enzyme is an RNA polymerase.

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25. The method of claims 24, wherein said inhibitor is a decoy probe comprising a nucleotide base recognition sequence region which binds to said RNA polymerase.

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26. The method of claims 24, wherein said inhibitor is a decoy probe comprising a nucleotide base recognition sequence having at least 35% sequence similarity to a promoter sequence recognized by said RNA polymerase sequence.

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27. A transcription-associated amplification procedure comprising the step of amplifying a target nucleic acid sequence to produce multiple copies of RNA transcripts by combining together under transcription-associated amplification conditions a mixture

comprising a target nucleic acid comprising said target nucleic acid sequence, a promoter-template complementary probe, a DNA polymerase, an RNA polymerase, ribonucleoside triphosphates, deoxyribonucleoside triphosphates, and means for reversibly inhibiting said RNA polymerase, wherein said means for reversibly inhibiting said RNA polymerase does  
5 not hybridize to a target nucleic acid comprising said target nucleic acid sequence under said amplification conditions to form a stable inhibitor:target complex.

28. The method of claim 27, wherein said DNA polymerase is a reverse transcriptase and said means for reversibly inhibiting said RNA polymerase is not a  
10 substrate in a primer extension reaction.

29. The method of claim 28, wherein said RNA polymerase, said reverse transcriptase, and said means for reversibly inhibiting said RNA polymerase are first combined together in the absence of said promoter-template complementary probe.  
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30. An improved method of amplifying a nucleic acid, wherein the improvement comprises the step of providing a nucleic acid polymerase with means for reversibly inhibiting said polymerase prior to providing said polymerase to said nucleic acid.  
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31. The method of claim 30, wherein said means for reversibly inhibiting said polymerase is not a substrate in a primer extension reaction.

32. The method of claim 31, wherein said amplifying is a transcription-associated amplification.  
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33. The method of claim 32, wherein said amplifying is a strand displacement amplification.

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